The flagellar type III protein export apparatus and F/V type ATPases share a common architecture

Bacteria swim in liquid environment using a helical filamentous organelle called the flagellum, which is composed of a rotary motor and an axial tubular structure. The flagellum is a large macromolecular assembly made of tens of thousands of almost 30 different protein molecules, most of which are exported to the distal end of the growing flagellum for self-assembly. The axial component proteins and the proteins that help flagellar assembly are efficiently translocated from the cytoplasm into the 2 nm central channel of the growing flagellum by the flagellar protein export apparatus [1]. The proteins involved in the flagellar protein export is highly homologous to those of the type III secretion system of pathogenic bacteria, which injects virulence effectors directly into their eukaryotic host cells for invasion. Thus the understanding of flagellar protein export will provide useful information for unveiling the mechanism of the virulence type III secretion system.

The flagellar export apparatus consists of the export gate made of six integral membrane proteins and three soluble proteins, FliH, FliI and FliJ [2]. These soluble components assemble onto and disassemble from the gate during the export cycle (Fig. 1). FliI is an ATPase that facilitates the flagellar protein export process, and FliH regulates the ATPase activity of FliI. FliI shows an extensive structural similarity to the α/β subunits of F_oF_1-ATP synthase [3]. FliI forms a homo hexamer ring just like the one formed by three α/β hetero dimers, which is the core part of F_1-ATPase. FliH was identified to have a sequence homology with the b and δ subunits of F_1-ATPase, which form the peripheral stalk connecting F_1 with F_o to function as the stator. FliJ is essential for efficient protein export but its function remains obscure. FliJ seems to regulate the entire export process through the dynamic interactions with its binding partners, because it interacts with various flagellar proteins. To elucidate the molecular mechanism of the flagellar protein export and the role of FliJ in the export process, we carried out the structural analysis of FliJ from *Salmonella enterica* serovar Typhimurium [4].

FliJ is a 17 kDa protein consisting of 147 residues. FliJ tends to form insoluble aggregates, but the addition of three residues, Gly-Ser-His, to its N-terminus as a His-tag stub prevented it from aggregation. This allowed us to crystallize FliJ for structure determination. The FliJ structure was solved at 2.1 Å resolution by using anomalous X-ray diffraction data from a mercury derivative crystal collected at beamline **BL41XU**.

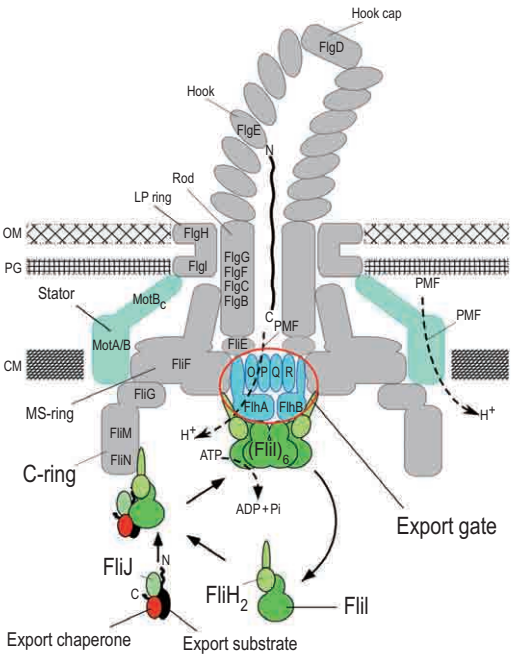

Fig. 1. Schematic diagram of the bacterial flagellar type III protein export apparatus. FlhA, FlhB, FliO, FliP, FliQ and FliR are integral membrane components and FliH, FliI and FliJ are cytoplasmic components. FliI forms a hetero-trimer with the FliH dimer in the cytoplasm and assembles into a hexamer upon docking to the gate with FliH and FliJ.

FliJ consists of two long α-helices that form an anti-parallel coiled-coil structure. This structure is remarkably similar to the coiled-coil region formed by the N- and C-terminal α-helices of the γ subunit of F_1-ATPase (Fig. 2). Although FliJ and the γ subunit have no apparent sequence similarity, structure-based sequence alignment revealed a conserved region between them. The conserved residues are localized on the surface where the ε subunit interacts with the γ subunit in F_1-ATPase, suggesting that FliJ may interact with a protein corresponding to the ε subunit in the export apparatus (Fig. 2).

The structural similarities between FliJ and the γ subunit and between FliI and the α/β subunits imply that FliJ and FliI may form a complex similar to F_1-ATPase. In fact, when FliI and FliJ were mixed at a molar ratio of 6:1 with Mg^{2+}-ADP-AlF_4, the FliI ring formation was much enhanced, suggesting that FliJ interacts with the FliI ring and facilitates the ring formation. The FliI and FliI-FliJ ring complexes,

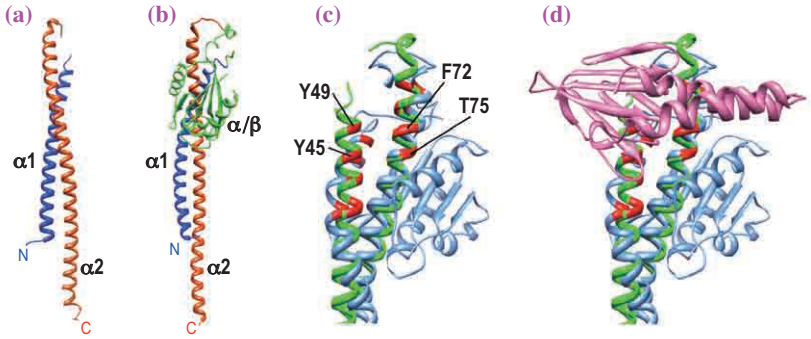

Fig. 2. Structural comparison of FliJ and the γ subunit of F_1-ATPase. Cα ribbon representation of **(a)** FliJ and **(b)** the γ subunit of F_1-ATPase. The α1 and α2 helices and the α/β domain of the γ subunit are colored blue, red and green, respectively. **(c) (d)** Close-up view of the conserved region between FliJ and the γ subunit. FliJ (green) is superimposed to the γ subunit (light blue). Residues conserved among FliJ homologs and γ subunits are shown in red. **(d)** The ε subunit (pink) is indicated with FliJ and the γ subunit. [4]

however, were not stable enough for crystallization work. We therefore analyzed the structures of the complexes by electron cryomicroscopy. Because the orientation of the ring particles embedded in vitreous ice was strongly biased to end-on views, we constructed 2D averaged images of the complexes and compared them. The averaged image of the FliI ring showed a hexameric ring structure with a central hole of 2 nm in diameter. On the contrary, the mixture of FliI and FliJ showed the same hexameric ring as the FliI ring but an extra density was identified in the central hole. These observations indicate that FliJ penetrates into the central hole of the FliI hexamer ring just like the γ subunit of F_1-ATPase (Fig. 3).

On the basis of these structures we proposed a model of the export apparatus (Fig. 4). The structural similarities found in this study suggest a similar mechanism and an evolutionary relationship between the type III protein export system and F- and V-type ATPases, despite that these two biological nanomachines have been thought to be totally unrelated to each other.

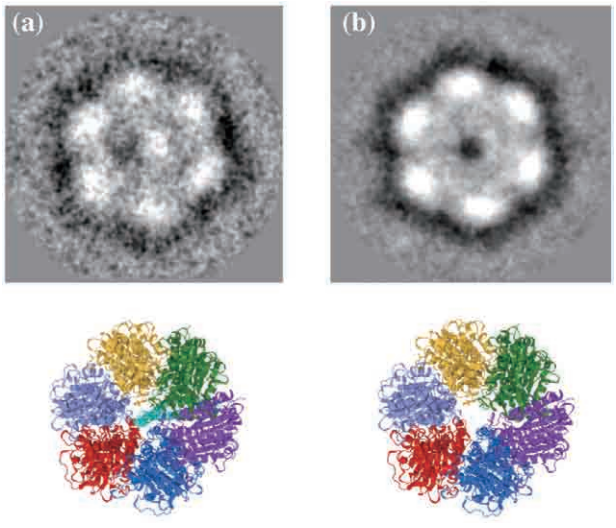

Fig. 3. Averaged cryoEM images of the FliI-FliJ **(a)** and FliI **(b)** ring complexes. Plausible ribbon models are indicated below each EM image.

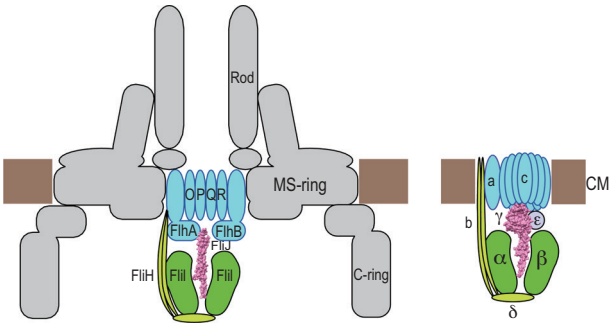

Fig. 4. Schematic diagram of a plausible model of the FliH-FliI-FliJ complex attached to the export gate. F_oF_1-ATP synthase is shown in the right panel as a reference. The flagellar basal body is colored gray. CM is the cytoplasmic membrane. [4]

Katsumi Imada[a,*], Tohru Minamino[b] and Keiichi Namba[b]

[a] Dept. of Macromolecular Science, Osaka University
[b] Dept. of Frontier Biosciences, Osaka University

*E-mail: kimada@chem.sci.osaka-u.ac.jp

References

[1] T. Minamino *et al.*: Mol. Biosyst. **4** (2008) 1105.
[2] T. Minamino and R.M. Macnab: J. Bacteriol. **181** (1999) 1388.
[3] K. Imada *et al.*: Proc. Natl. Acad. Sci. USA **104** (2007) 485.
[4] *T. Ibuki, K. Imada, T. Minamino, T. Kato, T. Miyata and K. Namba: Nat. Struct. Mol. Biol. **18** (2011) 277.*